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THE GERMINATION OF THE SPORES OF CONOCEPHALUM CONICUM

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INTRODUCTION

This work was undertaken in order to determine the time and the extent of the germination of the spores within the capsule of *Conocephalum conicum* (L.) Dumort.

In 1895 Farmer (3) stated that the spores of this species germinate before leaving the capsule, but that the divisions of the spore do not occur so early as in *Pellia*, in which plant germination sometimes occurs even before the separation of the four spores of a tetrad.

In 1903 Cavers (2) described the "mature spore" of *Conocephalum* as an ovoid mass of five or six cells.

In 1905 Bolleter (1) reported that the divisions of the spores begin in the spring and that the "mature spore" is many-celled.

MATERIAL AND METHODS

The plants used for these investigations were collected in the fall and spring of the years 1918-19 and 1919-20 on a bluff in almost the extreme southwestern corner of the state of Wisconsin about three miles southwest of Sinsinawa Mound.

The material used for the study of the spores and of their germination under normal conditions was collected beginning September 1, 1918, and continuing at intervals of not more than two weeks until December 20. The collections were resumed on March 7, 1919, and continued until April 17, at which time spore dispersal had already begun. The sporophytes were put into the killing fluid either in the field or immediately after being brought into the laboratory.

The most successful of the killing fluids used were Flemming's medium solution for the fall and early spring stages, and Flemming's strong solution for the later spring stages. The material was imbedded in 53° paraffin and microtome sections were made from seven to nine microns in thickness. All stages were studied from the fresh material as well as in prepared sections.

Immediately after dispersal, some of the sporelings¹ were sown in rain water in petri dishes and placed, some in a north exposure and others in a

¹ The multicellular structures resulting from the intra-capsular germination of the spores have been commonly referred to by previous writers as "spores" or "mature spores." They are, of course, strictly speaking, young gametophytes, and will be spoken of in the present paper as sporelings.

south exposure. Sporelings which were kept in a paper packet for thirty-six days after dispersal were sown on tap water in order to study the effect of drying on their power of further development.

In order to observe the effect of artificial conditions on the sporelings, gametophytes bearing carpocephala were collected in the latter part of October and on November 28, 1919. A spring collection was made on March 17, 1920, when the plants were still frozen. All the material when collected showed five, six, or seven cells in the sporelings, and was, so far as could be determined, in about the same condition as the material which was collected in October and November, 1918, and on March 7, 1919. The plants were placed under bell jars in a north room the temperature of which seldom exceeds 68° F. From time to time the plants were watered according as they showed signs of drying.

GERMINATION AND DEVELOPMENT UNDER NORMAL CONDITIONS

The spore mother cell is, as described by Farmer (3), a large, flattened, oval body rather the shape of a biscuit. The nucleus is large and, in the material studied, appeared as a homogeneous mass (Pl. XXXIV, fig. 1), showing that the spore mother cells were evidently in that stage previous to tetrad formation in which Farmer found the nucleus to take the stains in a similar manner.

The spores, still united in tetrads, were examined from the living material on September 15 and were easily discerned as three-faced pyramids enclosed within a thin mother cell wall. The stained preparations of this material were almost altogether unsatisfactory because of contraction in the cells (fig. 2). Farmer also reports this stage as being particularly difficult to fix satisfactorily.

As early as September 21, the spores were already freed from the mother cell wall although they were not yet fully rounded. Gradually they took on a somewhat globular form and the spore wall was differentiated into two layers, the outer one of which was golden brown in color and much dotted with tiny, bead-like projections. At the same time a large amount of starch was laid down in the spores and there was an increase in the size of the nucleus and of the spore itself (fig. 3).

Cell division in the spores was found as early as October 5, but this process did not go on simultaneously in every capsule of a single head, nor, indeed, in every spore within a given capsule. Some capsules were found wherein no divisions had occurred and others in which there were one-, two-, and four-celled sporelings (figs. 3, 4, 5).

According to Bolleter (1) the spore of *Conocephalum conicum* remains in the one-celled stage throughout the winter. Possibly climatic conditions are the cause of the difference between his results and mine, since the material studied by him was collected near Zürich. But since he records no collection later than the beginning of October, and none thereafter earlier

than the return of growing weather in March, the question arises as to whether the first divisions, which had already taken place in the fall, might have been thought by him to be spring divisions. The fact that the times stated for tetrad formation, stalk elongation, spore dispersal, and other activities of the plant, as observed by Bolleter, seem to be approximately the same as in material studied by me, makes it seem still more likely than his statement regarding the time of the first divisions of the spores is erroneous.

The partition walls when first formed between the cells of a sporeling are notably thin, and with Flemming's triple stain are scarcely discernible. Light green, when used instead of Orange G, however, gave them sufficient prominence to make them easily visible. After the first three divisions have occurred (fig. 6), there is a slight enlargement of the cells, a thickening of the partition walls, and a deposition of starch (fig. 7).

Material collected on March 7 showed no signs of change as compared with the late fall and winter collections, but on the 19th of March, the first really spring-like day, further divisions within the spore wall had taken place. Division stages were also observed in some of the preparations from this material. Within a few days the sporelings showed as many as ten or eleven cells in a median longitudinal section (fig. 8). Again there was a stoppage of cell division, and a period followed during which time there was growth and rapid development of chlorophyll within the cells. The next series of cell divisions took place early in April, and by April 9 the sporelings showed as many as seventeen cells in a median longitudinal section (fig. 9). It was noted that each period of cell division in the sporelings was preceded by growth and by the production and storage of starch.

During the latter part of this series of divisions, but more especially after the process was completed, the stalks of the carpocephala elongated rapidly until the sessile heads were raised five or six centimeters above the thallus. This rapid elongation is due, as is stated by Cavers (2) and Bolleter (1), to the growth of the cells already formed rather than to cell divisions within the stalks.

The sporelings which were sown immediately after dispersal almost without exception promptly resumed their development, and within less than twenty-four hours the majority showed at least two, and many of them showed three rhizoids (figs. 10, 11). A bud destined to develop into a thallus (*b*, fig. 12), usually appeared within four or five days, and a little later, secondary rhizoids were developed from the growing thallus (fig. 13). The above description holds for those sporelings which were placed in weak, as well as for those placed in strong, illumination. But in the later stages there was a relatively rapid and profuse development of both thalli and secondary rhizoids in the material which was placed in strong illumination (Pl. XXXV, figs. 14, 15).

To test the effect of drying, some of the sporelings which had been kept

in a paper packet for thirty-six days were sown on tap water and placed in moderate light. For a few days there seemed to be no development, though there was a significant distention and a marked suggestion of chlorophyll development in individual sporelings. No certain evidence of the development of thalli was observed in this culture for several days, but within a week or so, probably as many as ten percent of the sown sporelings began to develop further. However, the greater number of those sporelings which developed thalli formed no primary rhizoids (figs. 16, 17). This would seem to indicate that, as Cavers (2) and Bolleter (1) have reported, the cells which normally develop rhizoids are more susceptible to desiccation than are the other cells of the sporelings. A very small percentage of these dried sporelings, nevertheless, developed in an apparently perfectly normal way.

EFFECT OF ARTIFICIAL CONDITIONS

In the material collected in the fall, very little change was noted as late as December 15, when some of the sporelings of the collection made in November were sown on water. Sporelings from plants collected in late October, after being indoors nearly three months, were sown, but only a very small number of these showed a history of growth, cell division, and the development of rhizoids similar to that described below.

Of the sporelings collected in November, the majority developed after being sown. Many showed one primary rhizoid each, very few showed two, and some showed none (figs. 18, 19). The young thalli developed rapidly and all sent out secondary rhizoids, although the latter were proportionately few in number (figs. 20, 21) as compared with those of the thalli which developed from sporelings collected and sown in the spring (figs. 11, 15).

Toward the end of December, the stalks of the carpocephala of the plants collected in November began to elongate, though slowly, until they were about two centimeters in length. There was also a corresponding lengthening of the setae of the sporophytes. During a period of ten days or more, there was no further advance toward spore dispersal. The capsules ruptured the calytra and the enveloping sheath, but the setae did not elongate sufficiently to permit the ordinary dehiscence and sporeling dissemination. Some of the sporelings from these capsules were sown, however, and within less than thirty-six hours a few showed signs of development, and in time practically all of them developed as shown in figures 22-25.

The plants collected on March 17 showed signs of the elongation of the stalks of the carpocephala on the second day after being brought into the laboratory. The old gametophytes developed each a new thallus by means of growth from the apical region. After these plants had been in the laboratory for a week, the stalks had grown still further, until on April 2 some of them were as much as six centimeters in length. This was accompanied by the lengthening of the setae of the sporophytes, the rupturing

of the calypters, growth of the enveloping sheaths, the dehiscence of the capsules, and finally by an apparently normal dispersal of the sporelings.

An examination of these sporelings from time to time, previous to and following the elongation of the stalks of the carpocephala, showed no change in them until the stalks were about three centimeters in length. Then there occurred a slight increase in size of the sporelings, cell divisions, and good chlorophyll development. The sporelings apparently did not develop any further before their dispersal unless it was that there was more extensive chlorophyll development than before the divisions. At the time of dispersal the sporelings showed only 8 to 12 cells each, instead of 30 to 40 cells as is the case with the sporelings dispersed under natural out-of-door conditions. All these sporelings were still enclosed within the spore walls. Some of them were sown, and their subsequent development was almost as rapid as had been observed in those that were subjected to normal out-of-door conditions. The number of primary rhizoids, however, was never more than two, and in most cases there was but one to each sporeling (figs. 26, 28). The number of secondary rhizoids in each case was also small as compared with the number produced by thalli which grew from normally developed sporelings.

Cavers' (2) description of the mature "spores" of *Conocephalum conicum*, both as to the number of their cells and the number of primary rhizoids developed subsequent to their being sown, is very similar to my observations just detailed on plants which had been subjected to artificial conditions. It seems possible, therefore, that the plants which he studied were also subjected to other than perfectly natural conditions.

SUMMARY

1. The spore mother cells of *Conocephalum conicum* are well developed in this region before September first, and the spores are freed from the spore mother cell walls about the middle of September. Toward the end of the month the spores are well-rounded, rough-walled, and each contains a relatively large nucleus.

2. In the early part of October cell division begins, this being preceded by growth and by a heavy deposit of starch. Before the middle of the month as many as five cells are seen in a median longitudinal section, so that there are probably as many as six to eight cells in each sporeling. These cells all remain within the spore wall.

3. Before winter sets in, there is a thickening of the partition walls, a deposition of starch, and growth in the cells formed by the division of the spores. In this condition the usually six- to eight-celled sporelings rest through the winter.

4. Cell division is resumed with the coming of the first warm weather in the spring and proceeds rapidly until the stored food is consumed. Then, during a pause in cell division, there is growth of the cells, a rapid

development of chlorophyll and of starch, followed by a second series of cell divisions until each sporeling has developed into a nearly spherical mass of from thirty to forty cells.

5. A short time before the cell divisions are complete the stalks of the carpocephala begin to lengthen, and during four or five days after the sporelings have matured, these stalks elongate rapidly until they attain a height of five or six centimeters.

6. Simultaneous with the rapid elongation of the stalks is a lengthening of the setae of the sporophytes by means of which the capsule is thrust through the calyptra and the enveloping sheath. The capsule wall is then ruptured and the sporelings and elaters are dispersed.

7. The sporelings are on the whole rather short-lived, though some are capable of developing thalli after being dried for as long as thirty-six days.

8. The sporelings collected in the late fall develop subsequent to their being sown even though there are no stalk elongation, no spore dispersal, and no cell divisions previous to the sowing after those which occurred under natural conditions.

9. The stalks of the carpocephala, which normally begin elongation after a series of spring divisions in the cells of the sporelings, elongate before such divisions occur if the plants are brought indoors.

10. The number of cells in a naturally developed sporeling before dispersal is from thirty to forty, while the number of cells in the sporeling which has been subjected to artificial conditions is from five to twelve according as these conditions more or less closely approach the natural ones.

11. The number of primary rhizoids produced by a normal sporeling ranges from three to five, while the number from the sporeling placed under artificial conditions is never more than two, most often only one, and in some cases no primary rhizoid is produced. The number of secondary rhizoids from the thalli developed from sporelings subjected to artificial conditions is also relatively few as compared with the number developed from the thalli of naturally developed sporelings.

I wish to express my sincere thanks to Professor C. E. Allen who directed the writing of this paper, for his many helpful suggestions and criticisms, and to Dr. W. N. Steil who suggested the work, for his encouragement and assistance during its progress.

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EXPLANATION OF PLATES

All figures were made with the aid of a camera lucida and drawn at table level. Leitz objective no. 6 and ocular no. 4 were used in making figures 1-9; objective no. 3 and ocular no. 4 were used in drawing figures 10-17. With a tube length of 170 mm., a magnification of about 660 was obtained in figures 1-9 and a magnification of 140 in figures 10-17. For figures 18-28 a Bausch & Lomb 16 mm. objective and 12.5 mm. ocular gave a magnification of 200. These magnifications were reduced by one-third in reproduction.

The following abbreviations are used: *r*, rhizoid; *b*, bud; *s*, sporeling; *y t*, young thallus; *s c*, secondary rhizoid.

PLATE XXXIV

FIG. 1. A spore mother cell.
 FIG. 2. Spore tetrad within the mother cell wall.
 FIG. 3. Spore just before its first division.
 FIG. 4. Two cells formed by the division of a spore and remaining surrounded by the spore wall.
 FIG. 5. Four cells formed within the spore wall.
 FIG. 6. Median longitudinal section of a sporeling showing five cells. Probably as many as eight cells are present.
 FIG. 7. Same as figure 6, after the thickening of the walls and the deposition of starch.
 FIG. 8. Condition of sporeling during the first series of spring divisions.
 FIG. 9. Median longitudinal section of a sporeling ready for dispersal.
 FIG. 10. First stage in external development. Sporeling twelve hours after being sown on rain water.
 FIG. 11. Sporeling about twenty-four hours after being sown on rain water.
 FIG. 12. Later stage showing the development of a bud (*b*) which is to grow into a thallus.
 FIG. 13. Gametophytes about six days old showing secondary rhizoids.

PLATE XXXV

FIGS. 14, 15. Gametophytes developed in strong light.
 FIGS. 16, 17. Sporeling which had suffered desiccation before being sown. No rhizoids have developed.
 FIGS. 18, 19. Developing sporelings which were removed from the capsule before there was any advance toward spore dispersal.
 FIGS. 20, 21. Later stages in the development of gametophytes treated like those shown in figures 18 and 19.
 FIGS. 22-25. Stages in the external development of sporelings from the same collection as those shown in figures 18 to 21, but which were not sown until after there was some elongation of the stalks of the carpocephala and of the setae of the sporophytes.
 FIGS. 26-28. Stages in the external development of sporelings subjected to indoor conditions beginning March 7, and after which they were dispersed naturally.



